(iv) Its ultraviolet absorption spectrum is characteristic of a conjugated heptaene and is qualitatively the same as that of the candicidin working standard.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve a portion of the sample in sufficient dimethylsulfoxide to yield an estimated concentration of 1,000 micrograms of candicidin activity per milliliter. Further dilute an aliquot with sterile distilled water to the reference concentration of 0.06 microgram of candicidin activity per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using a 1 percent aqueous suspension.

(4) *Identity*—(i) *Preparation of aqueous alcohol solution.* Prepare an aqueous alcohol solution by mixing 53 volumes of ethyl alcohol and 47 volumes of water.

(ii) *Preparation of standard solution.* Grind a small portion of the candicidin working standard to a fine powder with a mortar and pestle. Accurately weigh an amount equivalent to 20,000 micrograms of candicidin activity and transfer it to a 100-milliliter volumetric flask. Add about 50 milliliters of the aqueous alcohol solution and shake to effect complete dissolution. Bring to volume with the aqueous alcohol solution and mix well. Transfer a 25-milliliter aliquot to a 100-milliliter volumetric flask and bring to volume with the aqueous alcohol solution. This solution contains 50 micrograms of candicidin activity per milliliter.

(iii) *Preparation of sample solution.* Proceed as directed in paragraph (b)(4)(ii) of this section.

(iv) *Procedure.* Using a suitable recording spectrophotometer, record the absorption spectra of the standard solution and the sample solution between the wavelengths of 330 and 410 nanometers with the aqueous alcohol solution as the reference solution. Compare the absorption spectra of the standard solution and the sample solution. They should exhibit absorption maxima and minima at the same wavelengths, which are approximately 342, 359, 378, and 397 nanometers for the maxima and 348, 366, and 390 nanometers for the minima.

[39 FR 19134, May 30, 1974, as amended at 44 FR 30333, May 25, 1979; 49 FR 2243, Jan. 19, 1984]

§ 449.20 Griseofulvin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Griseofulvin is a microsize, white to pale-cream compound with the following chemical name: 7-chloro-2′,4,6-trimethoxy-6′β-methylspiro[benzofuran-2(3*H*),1′-[2]cyclohexene]-3,4′-dione. It is so purified and dried that:

(i) Its griseofulvin content is not less than 900 micrograms and not more than 1,050 micrograms of griseofulvin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 1.0 percent.

(iv) Its melting point, after drying, is not less than 217° C. and not more than 224° C.

(v) Its specific rotation in dimethylformamide at 25° C. is not less than +348° and not more than +364°.

(vi) Its ultraviolet absorption spectrum in methyl alcohol compares qualitatively with that of the griseofulvin reference standard.

(vii) Its residue on ignition is not more than 0.2 percent.

(viii) Its heavy metals content is not more than 25 parts per million.

(ix) Its specific surface area is not less than 1.3 and not more than 1.7 square meters per gram.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for griseofulvin content, loss on drying, melting point, specific rotation, identity, residue on ignition, heavy metals, specific surface area, and crystallinity.

(ii) Samples required: 10 packages, each containing not less than 1 gram.

(b) *Tests and methods of assay*—(1) *Griseofulvin content (gas liquid chromatography).* Proceed as directed in § 436.321 of this chapter.

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *Melting point.* Proceed as directed in § 436.209 of this chapter.

(5) *Specific rotation.* Accurately weigh approximately 250 milligrams of the sample in a 25-milliliter glass-stoppered volumetric flask and dissolve in about 15 milliliters of dimethylformamide. Bring to volume with dimethylformamide, stopper, and mix well. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube.

(6) *Identity.* Dissolve an accurately weighed portion of the sample and of the griseofulvin working standard and dissolve each in sufficient methyl alcohol to obtain a concentration of 10 micrograms of griseofulvin per milliliter and mix well. (The standard solution can be kept under refrigeration and used for up to 1 month.) Record the ultraviolet absorption spectrum of solutions of the sample and standard from 240 to 320 nanometers. The spectral curves shall be similar, and each shall have a maximum at 292± 2 nanometers and a minimum at 269± 2 nanometers.

(7) *Residue on ignition.* Proceed as directed in § 436.207 of this chapter.

(8) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(9) *Specific surface area*—(i) *Procedure.* Determine the apparent particle size in microns by the air-permeation method, using a suitable subsieve sizer. Weigh 1.819 grams ±0.001 gram of the sample and transfer to the compression tube of the apparatus. Compact the sample with moderate pressure so that it has a uniform porosity. Pass compressed dry air through the sample and measure the air pressure with a water manometer. Observe the porosity and calculate the apparent particle size from the instrument equation or read it from a chart that has been calculated in accordance with the equation. Repeat the readings at successively higher degrees of compaction until the apparent particle size reaches a minimum. Calculate the observed specific surface area (SSA) in square meters per gram of sample, as follows:

$$\text{Observed SSA} = \frac{6}{\text{Minimum apparent particle size in microns} \times 1.455 \times F}$$

where F is a factor used to correct the apparent particle size to the true particle size:

Porosity reading	F
0.80	1.3771
0.76	1.4142
0.72	1.4573
0.68	1.5082
0.64	1.5690
0.60	1.6432
0.56	1.7353
0.52	1.8528
0.48	2.0076
0.44	2.2203
0.40	2.5298

(ii) *Standard.* Determine the observed specific surface area of the griseofulvin specific surface area standard by the method prescribed in paragraph (b)(9)(i) of this section, using the same instrument and the same air pressure setting.

(iii) *Calculations.* Calculate the corrected specific surface area of the sample as follows:

$$\text{SSA of sample} = \frac{\text{Observed SSA of sample} \times \text{assigned SSA of standard}}{\text{Observed SSA of standard}}$$

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19134, May 30, 1974, as amended at 44 FR 20660, Apr. 6, 1979; 50 FR 19920, May 13, 1985]

§ 449.40 Natamycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Natamycin is 22-[(3 - amino - 3,6 - dideoxy -β-D-mannopyranosyl) - oxy] - 1,3,26-trihydroxy - 12-methyl-10-oxo-6,11,28-
trioxatricyclo [22.3.1.$0^{5,7}$] octacosa-8,14,16,18,20 - pentaene-25-carboxylic acid. It is an off-white to cream colored powder which may contain up to 3 moles of water. It is practically insoluble in water, slightly soluble in methanol, and soluble in glacial acetic acid and dimethylformamide. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of natamycin per milligram on an anhydrous basis.

(ii) Its moisture content is not less than 6.0 percent and not more than 9.0 percent.

(iii) Its pH in a 1 percent aqueous suspension is not less than 5.0 and not more than 7.5.

(iv) It passes the identity test.

(v) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay.* Dilute solutions of natamycin are very sensitive to light and should be kept in the dark as much as possible or substantial decomposition will take place.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in dimethylsulfoxide and further dilute with sufficient dimethylsulfoxide to give a concentration of 100 micrograms of natamycin per milliliter (estimated). Further dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 5.0 micrograms of natamycin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using a 1.0 percent aqueous suspension.

(4) *Identity.* Accurately weigh approximately 50 milligrams of the sample into a 200-milliliter volumetric flask. Add approximately 5.0 milliliters of distilled water, and completely moisten the sample. Then add approximately 100 milliliters of an acid-alcohol solvent (0.1 percent glacial acetic acid in methyl alcohol) and stir or shake mechanically in the dark until solution is complete. Dilute to volume with the acid-alcohol solvent. Transfer 2.0 milliliters of this solution to a 100-milliliter volumetric flask and dilute to volume with the acid-alcohol solvent. Using a suitable spectrophotometer with 1-centimeter cells and the acid-alcohol solvent as a blank, record the ultraviolet absorption spectrum from 215 to 330 nanometers. The spectrum compares qualitatively to that of the natamycin working standard similarly treated.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[43 FR 55384, Nov. 28, 1978, as amended at 46 FR 16684, Mar. 13, 1981]